



Attorney Docket No.: P-514 (TI-0011)
Inventors: Paul D. Taylor
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This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes.

Claim 2 (original): A method of claim 1 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 said mobile phase comprising:

- an eluting salt composed of equal concentrations of:
 - a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or

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mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5, and,

an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M,

Claim 3 (original): A method of claim 2 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

Claim 4 (original): A method of claim 2 wherein said cation is selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, wherein the alkyl groups consist of any combination of methyl and ethyl.

Claim 5 (currently amended): A method of claim 2 chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

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(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, and contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 said mobile phase comprising an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl and an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof;

(c) a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and

(d) an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M, and wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes and wherein said cation comprises choline.

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Claim 6 (original): A method of claim 2 wherein said cation comprises guanidinium.

Claim 7 (original): A method of claim 2 where said cation comprises sodium.

Claim 8 (original): A method of claim 2 wherein said anion is formate or chloride.

Claim 9 (original): A method of claim 2 wherein said mobile phase includes a metal chelating agent.

Claim 10 (currently amended): A method of claim 9 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, ~~α -furildioxime~~, nioxime, salicylaloxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α' , α'' -

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terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium diethyldithiocarbarbamate, zinc, dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

Claim 11 (original): A method of claim 1, wherein said solid is comprised of a silica, polysaccharide or synthetic polyolefin backbone.

Claim 12 (original): A method of claim 11 wherein said polyolefin is a polystyrene or polyacrylic.

Claim 13 (original): A method of claim 1, wherein said solid comprises a polyacrylic backbone.

Claim 14 (original): A method of claim 1, wherein said solid comprises diethylaminoethyl functional groups.

Claim 15 (original): A method of claim 1, wherein said solid comprises polyethyleneimine functional groups.

Claim 16 (original): A method of claim 1, wherein said solid comprises particles with an average diameter between approximately 2 micron and 10 micron.

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Claim 17 (original): A method of claim 1, wherein the solid is substantially nonporous.

Claim 18 (original): A method of claim 1, wherein said solid comprises a polystyrene backbone.

Claim 19 (original): A method of claim 1, wherein said mobile phase contains an organic solvent selected from the group consisting of methanol, ethanol, acetonitrile, ethyl acetate, formamide, 2-propanol, and N-methyl pyrrolidone.

Claim 20 (original): A method of claim 1 wherein said mobile phase contains less than about 40% by volume of said organic solvent.

Claim 21 (original): A method of claim 1 wherein said eluting is carried out at a column temperature greater than about 50°C.

Claim 22 (original): A method of claim 1 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claim 23 (original): A method of claim 1 wherein the concentration of said eluting salt is continuously increased.

Claim 24 (original): A method of claim 1 including analyzing the mobile phase after the elution step (b) for the concentration of said DNA molecules.

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Claim 25 (original): A method of claim 24 wherein the concentration of said DNA molecules is measured by ultraviolet absorbance in the approximate wavelength range of about 250nm to about 290nm.

Claim 26 (original): A method of claim 1 wherein the total time required to complete said method is between about 2 minutes and about 30 minutes.

Claim 27 (original): A method of claim 1 wherein the concentration of organic solvent is systematically increased.

Claim 28 (currently amended): A method of claim 1 where said solid is contained in a column of cylindrical ~~geomtery~~ geometry.

Claim 29 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

- (a) applying the mixture to an anion-exchange solid,
- (b) eluting the solid of step (a) with a mobile phase containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes.

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Claim 30 (currently amended): A method of claim 29 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5 ;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claims 31-32 (canceled).

Claim ³¹/₃₃ (currently amended): ~~A method of claim 30 chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture comprising :~~

(a) applying the mixture to an anion-exchange solid.

(b) eluting the solid of step (a) with a mobile phase

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containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes and further solid of step(a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium, and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl;

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M and wherein said cation comprises choline.

Claim ³²~~34~~ (original): A method of claim 30 wherein said cation comprises sodium

Claim ³³~~35~~ (original): A method of claim 30 wherein said mobile phase includes a metal chelating agent.

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Claim ~~36~~ (currently amended): A method of claim 35 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, ~~α -furildioxime~~, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitros-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α' , α'' -terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium ~~diethyldithiocarbamate~~ diethyldithiocarbamate, zinc dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

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Claim ~~37~~³⁵ (original): A method of claim 30 wherein said cation comprises guanidinium.

Claim ~~38~~³⁶ (original): A method of claim 30 wherein said anion is formate or chloride.

Claim ~~39~~³⁷ (original): A method of claim 30 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

Claim ~~40~~³⁸ (original): A method of claim 30 including analyzing the mobile phase eluting from the column for the presence of DNA.

Claim ~~41~~³⁹ (original): A method of claim 30 wherein said eluting is carried out at a column temperature greater than about 50°C.

Claim ~~42~~⁴⁰ (original): A method of claim 30 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claims 43-63 (canceled).

Claim ~~64~~⁴¹ (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer,

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wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising of equal concentrations of:

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim 65 (canceled).

Claim ⁴²~~66~~ (original): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

(a) applying the mixture to an anion-exchange solid;

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(b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising an eluting salt comprising:

betaine at a concentration in the range of about 0.5M to about 6M;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and,

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim ⁴³~~61~~ (original): A method of claim ⁴²~~66~~ wherein the eluting is carried out at a column temperature greater than about 50°C.

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Claim ~~68~~ (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising equal concentrations of :

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and wherein the eluting is carried out at a column temperature greater than about 50°C,

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

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Claim 69-71 (canceled).

Claim ~~72~~⁴⁵ (original): The method of claim 1, where prior to said applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules.